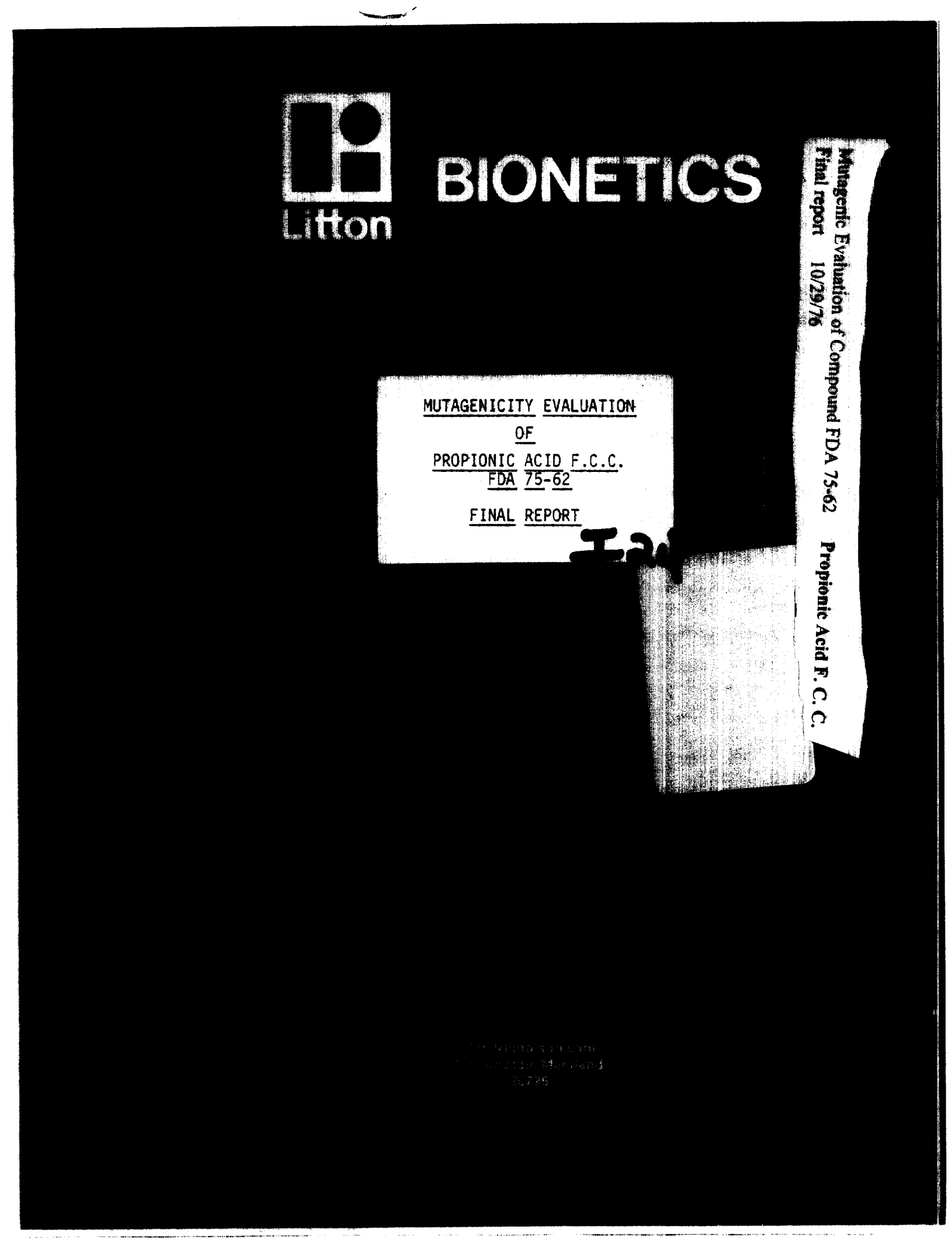
Litton
BIONETICS
Mutagenic Evaluation of Compound FDA 75-62
Final report 10/29/76
Propionic Acid F. C. C.
MUTAGENICITY EVALUATION
OF
PROPIONIC ACID F.C.C.
FDA 75-62
FINAL REPORT

MUTAGENICITY EVALUATION
OF
PROPIONIC ACID F.C.C.
FDA 75-62

FINAL REPORT

SUBMITTED TO

FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
5600 FISHERS LANE
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SUBMITTED BY

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LBI PROJECT NO. 2672

OCTOBER 29, 1976

Litton BIONETICS

TABLE OF CONTENTS

EVALUATION SUMMARY

The test compound Propionic Acid F.C.C., FDA 75-62, 000079094, did not exhibit mutagenic activity in any of the assays employed in these studies.

DATE: October 29, 1976

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound Propionic Acid F.C.C., FDA 75-62

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: September 3, 1976
2. Description: colorless liquid

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: *Saccharomyces cerevisiae*, strain D4

Bacteria Strains: *Salmonella typhimurium*, strains TA-1535
TA-1537
TA-1538
TA-98
TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

Component	Final Concentration/ml
1. TPN (sodium salt)	4 µmoles
2. Glucose-6-Phosphate	5 µmoles
3. Sodium Phosphate (dibasic) pH 7.4	100 µmoles
4. $MgCl_2$	8 µmoles
5. KCl	33 µmoles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS[b]
	Ethylmethanesulfonate	Water or saline	BPS[b]
	2-Nitrofluorene	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard	Water or saline	FS[b]
Activation	Dimethylnitrosamine	Water or saline	BPS[b]
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS[b]
	8-Aminoquinoline	Dimethylsulfoxide[c]	FS[b]
	2-Aminoanthracene	Dimethylsulfoxide[c]	BPS[b]

[a] Concentrations given in the Results Section
[b] BPS = base-pair substitution; FS = frameshift
[c] Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15 M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.

2. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: 000079-09-4

2. Test solvent: Saline

3. Solubility of the test compound under treatment conditions:
Soluble

4. Additional comments: Colorless liquid

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: September 8, 1976

2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

	Percent Concentration	
Test Doses	Bacteria	Yeast
1/4 50% Survival	0.02375	0.625
1/2 50% Survival	0.04750	1.250
50% Survival	0.09500	2.500

C. Suspension Assay Results

The suspension test results for the test compound are summarized in the following six tables. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second through the fourth table of the suspension set presents the results for the activation assays. The fifth table shows the results of the nonactivation plate test and the sixth table shows the results of the activation plate test. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control for ACP A-C = Activation: Solvent Control ALI or A+T = Activation: Homogenate Control (Liver) ALU = Activation: Homogenate Control (Lung) ACP = Activation: Positive Control ACT = Activation Test LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = x 10^6).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (*Macaca mulatta*)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES / NONACTIVATION COMPOUND 000079094

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
NAN		66.63	18.29	14.48	1.21	13.86	22.36	9.75	
NAP		729.93	4938.27	95.62	143.90	820.41	68.71	38.10	CONTROLS
NA1		69.50	9.34	13.53	1.93	10.92	14.45	9.87	
NA2		93.98	10.50	11.20	1.45	8.33	8.95	20.17	TEST DATA
NA3		82.69	13.72	22.09	1.41	5.60	10.50	7.91	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES ICRFLO/MOUSE COMPOUND 000079094

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	20.58	7.58		2.69	29.04	4.72		23.27	8.14	
ACT	A-C	22.30	5.57		5.85	28.54	3.09		26.15	7.98	
ACT	ALI	24.42	6.44	4.74	6.95	49.71	9.94	17.81	26.53	8.68	NEGATIVE CONTROLS
ACT	ALU	21.77	7.57	3.44	3.65	25.54	5.41	15.04	28.89	8.63	
ACT	PLI	70.14	136.26		142.77	202.67	106.30		67.69	27.36	
ACT	PLU	22.31	27.00		2.14	38.36	110.39		32.40	11.43	POSITIVE CONTROLS
ACT	LI1	20.01	50.62	3.52	13.97	29.20	18.77		24.31	8.37	
ACT	LI2	24.49	31.22	3.61	11.24	37.74	36.08	23.96	24.37	7.62	
ACT	LI3	24.61	14.66		6.98	24.89	13.76		17.74	7.10	TEST DATA
ACT	LU1	25.36	19.40	4.43	9.45	22.83	21.37	22.11	20.60	8.02	
ACT	LU2	30.06	6.69		8.54	36.64	10.80		21.99	9.59	
ACT	LU3	23.35	16.67		9.33	24.31	7.42		24.56	10.11	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES SPRDAW/RAT COMPOUND 000079094

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	20.72	10.78	4.17	4.92	15.89	43.00	21.56	
ACT	A-C	25.83	10.16	2.65	4.12	12.44	51.25	20.37	
ACT	ALI	31.45	13.54	3.74	18.45	11.62	41.81	26.41	NEGATIVE CONTROLS
ACT	ALU	27.04	12.64	2.11	7.49	20.06	39.12	21.07	
ACT	PLI	61.73	246.86	122.50	218.59	84.05	79.89	60.49	
ACT	PLU	28.22	13.97	1.37	273.20	24.85	40.92	22.52	POSITIVE CONTROLS
ACT	LI1	32.18	35.94	8.74	6.85	29.88	46.60	15.64	
ACT	LI2	31.87	21.83	8.04	21.43	26.31	42.59	14.53	
ACT	LI3	25.23	12.05	3.87	15.08	23.66	51.06	15.82	
ACT	LU1	35.76	5.65	7.76	7.07	23.81	40.50	14.77	TEST DATA
ACT	LU2	25.77	5.93	4.19	5.68	20.18	61.39	18.43	
ACT	LU3	29.08	4.72	2.30	6.85	30.23	47.04	11.84	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 10/27/76

SPECIES RHESUS/MONKEY COMPOUND 000079094

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	26.73	7.45	11.79	3.31	26.43	13.21	7.50	
ACT	A-C	25.84	9.64	7.98	6.21	27.12	16.19	7.53	
ACT	ALI	30.06	6.89	18.45	10.77	56.55	15.71	7.72	NEGATIVE CONTROLS
ACT	ALU	28.62	7.57	22.19	3.79	61.49	8.72	5.72	
ACT	PLI	60.22	58.15	3.07	580.84	88.51	67.41	24.55	
ACT	PLU	30.95	8.82	13.13	3.86	42.92	12.93	6.12	POSITIVE CONTROLS
ACT	LI1	28.84	8.24	22.58	8.84	29.61	14.33	8.54	
ACT	LI2	31.71	6.65	31.86	11.93	42.13	14.27	7.84	
ACT	LI3	30.55	7.30	24.68	15.43	42.03	13.38	7.30	
ACT	LU1	29.60	8.56	28.52	7.02	30.19	10.23	6.00	TEST CONTROLS
ACT	LU2	24.88	7.22	46.94	5.93	33.85	12.45	7.09	
ACT	LU3	31.47	6.73	36.78	2.82	28.44	10.74	7.94	

SUMMARY OF TEST RESULTS

PLATE TESTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 000079094

B. TEST DATE: OCT. 12, 1976

TEST		SPECIES	TISSUE	REVERTANTS PER PLATE									
				TA-1535		TA-1537		TA-1538		TA-98		TA-100	
				1	2	1	2	1	2	1	2	1	2
1. NON-ACTIVATION													
SOLVENT CONTROL*		---	---	31	23	31	19	19	18	22	21	201	248
POSITIVE CONTROL**		---	---	>1000	>1000	895	461	>1000	>1000	>1000	>1000	>1000	>1000
TEST	0.09500 %	---	---	13	17	15	14	21	14	36	28	315	189
	0.04750 %	---	---	18	16	25	20	17	16	40	22	297	216
	0.02375 %	---	---	29	17	23	26	12	27	32	35	286	206
2. ACTIVATION													
SOLVENT CONTROL*		MOUSE	LIVER	25	40	20	12	22	23	24	19	111	123
		RAT	LIVER	20	20	14	11	32	28	40	59	89	77
		MONKEY	LIVER	16	41	12	6	22	36	51	60	57	71
POSITIVE CONTROL***		MOUSE	LIVER	202	154	303	516	>1000	>1000	167	129	123	100
		RAT	LIVER	94	91	>1000	127	462	500	239	173	154	181
		MONKEY	LIVER	513	375	80	119	>1000	>1000	142	183	167	285
TEST	0.09500 %	MOUSE	LIVER	22	23	22	20	14	25	24	29	125	120
	0.04750 %	MOUSE	LIVER	39	32	21	23	20	18	39	37	135	101
	0.02375 %	MOUSE	LIVER	35	43	21	12	20	20	26	24	124	114
	0.09500 %	RAT	LIVER	21	24	18	19	22	17	43	48	72	75
	0.04750 %	RAT	LIVER	25	35	11	13	25	17	43	59	81	69
	0.02375 %	RAT	LIVER	31	40	18	14	20	18	45	54	62	77
	0.09500 %	MONKEY	LIVER	34	19	11	8	28	20	46	55	55	54
	0.04750 %	MONKEY	LIVER	17	23	15	7	17	17	53	56	56	83
	0.02375 %	MONKEY	LIVER	26	29	6	14	21	12	51	52	65	58

* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

** TA-1535 MNNG 2 UG/PLATE
TA-1537 QM 20 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 MNNG 2 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMQ 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

VI. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound: Propionic Acid F.C.C., FDA 75-62, 000079094.

A. Salmonella typhimurium

1. Plate Tests

The results of these tests were all negative.

2. Nonactivation Suspension Tests

The results of these tests were negative.

3. Activation Suspension Tests

The results of these tests were negative. LI_1, LI_2, and LU_2 doses with TA-1535 and LI_2 and LU_1 doses with TA-98 using mouse tissue were repeated because of increased mutant frequencies. The repeat tests were negative.

B. Saccharomcyes cerevisiae

1. Nonactivation Suspension Tests

The results of these tests were negative.

2. Activation Suspension Tests

The results of these tests were negative.

C. Conclusions

The test compound Propionic Acid F.C.C., FDA 75-62, 000079094, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

David J. Brusick, Ph.D. Date
Director
Department of Genetics

Reviewed by:

Robert J. Weir, Ph.D. Date
Vice President

VII. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagens to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical which produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical which produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test which cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

VIII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the *his*$^-$ cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.

C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

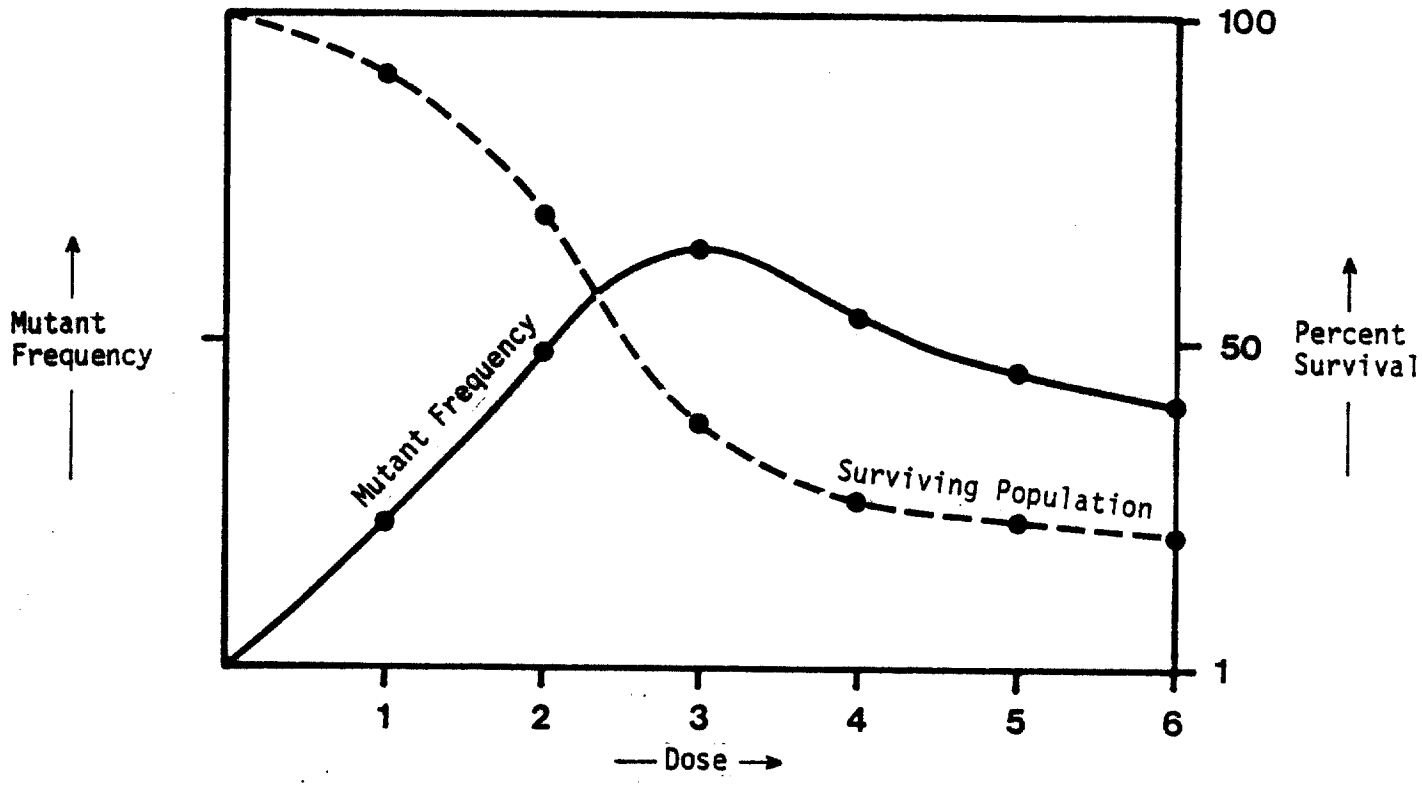
D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT	OBSERVED DOSE RESPONSE
(1) Dose levels 1,2 & 3 were used	A typical positive dose response set of data would be obtained.
(2) Dose levels 2, 3 & 4 were used	The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.
(3) Dose levels 3, 4 & 5 were used	Here an inverted dose response would be observed with the highest dose level showing the lowest response.

STANDARD OPERATING PROCEDURES

To ensure an accurate and reliable mutagenicity testing program, LBI instituted the following procedures:

- The test compound was registered in a bound log book recording the date of receipt, complete client identification, physical description and LBI code number.

- Complete records of weights and dilutions associated with the testing of the submitted material were entered into a bound notebook.

- Raw data information was recorded on special printed forms that were dated and initialed by the individual performing the data collection at the time the observations were made. These forms were filed as permanent records.

- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.

APPENDIX

Tabulation of Data

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 627205 DETECTOR TA100 SPECIES / DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0803	0535	66.63	0
	NAP		EMS 0.066%	0548	4000	729.93	0
000079094	NA1		0095-3 PCT.	0813	0565	69.50	0
000079094	NA2		0475-4 PCT.	0615	0578	93.98	0
000079094	NA3		2375-5 PCT.	0780	0645	82.69	0

REPORT EXR33 - LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 625801 DETECTOR TA1535 SPECIES / DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0257	0047	18.29	0
	NAP		EMS 0.2%	0081	4000	4938.27	0
000079094	NA1		0095-3 PCT.	0257	0024	9.34	0
000079094	NA2		0475-4 PCT.	0362	0038	10.50	0
000079094	NA3		2375-5 PCT.	0277	0038	13.72	0

REPORT EXR33, LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 626501 DETECTOR TA1537 SPECIES / DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0221	0032	14.48	0
	NAP		QM 13 UG/ML	0251	0240	95.62	0
000079094	NA1		0095-3 PCT.	0207	0028	13.53	0
000079094	NA2		0475-4 PCT.	0259	0029	11.20	0
000079094	NA3		2375-5 PCT.	0172	0038	22.09	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 626402 DETECTOR TA1538 SPECIES / DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0911	0011	1.21	0
	NAP		NF 667 UG/ML	0410	0590	143.90	0
000079094	NA1		0095-3 PCT.	0519	0010	1.93	0
000079094	NA2		0475-4 PCT.	0688	0010	1.45	0
000079094	NA3		2375-5 PCT.	0778	0011	1.41	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104
EXPERIMENT 625905 DETECTOR TA98 SPECIES / PROJECT 02468

DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0202	0028	13.86	0
	NAP		NF 667 UG/ML	0049	0402	820.41	0
000079094	NA1		0095-3 PCT.	0348	0038	10.92	0
000079094	NA2		0475-4 PCT.	0360	0030	8.33	0
000079094	NA3		2375-5 PCT.	0375	0021	5.60	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 628801 DETECTOR 00004 SPECIES / DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	1118	0250	0109	22.36	9.75	0
	NAP		EMS 1.0 %	0294	0202	0112	68.71	38.10	0
000079094	NA1		0025-1 PCT.	1266	0183	0125	14.45	9.87	0
000079094	NA2		0125-2 PCT.	1408	0126	0284	8.95	20.17	0
000079094	NA3		0625-3 PCT.	1428	0150	0113	10.50	7.91	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 627805 DETECTOR TA100 SPECIES ICRFLO/MOUSE DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	2342	0482	20.58	0
	A-C		SOLVENT	2274	0507	22.30	0
	ALI		TISSUE	2604	0636	24.42	0
	ALU		TISSUE	2104	0458	21.77	0
	ACP	LI	DMN 90 UM/ML	1400	0982	70.14	0
	ACP	LU	DMN 90 UM/ML	2506	0559	22.31	0
000079094	ACT	LI1	0095-3 PCT.	2654	0531	20.01	0
000079094	ACT	LI2	0475-4 PCT.	2160	0529	24.49	2
000079094	ACT	LI3	2375-5 PCT.	2202	0542	24.61	0
000079094	ACT	LU1	0095-3 PCT.	1928	0489	25.36	0
000079094	ACT	LU2	0475-4 PCT.	1856	0558	30.06	0
000079094	ACT	LU3	2375-5 PCT.	2544	0594	23.35	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 625904 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0488	0037	7.58	0
	A-C		SOLVENT	0539	0030	5.57	0
	ALI		TISSUE	0357	0023	6.44	0
	ALU		TISSUE	0383	0029	7.57	0
	ACP	LI	DMN 90 UM/ML	0353	0481	136.26	0
	ACP	LU	DMN 90 UM/ML	0337	0091	27.00	0
000079094	ACT	LI1	0095-3 PCT.	0405	0205	50.62	0
000079094	ACT	LI2	0475-4 PCT.	0410	0128	31.22	0
000079094	ACT	LI3	2375-5 PCT.	0348	0051	14.66	0
000079094	ACT	LU1	0095-3 PCT.	0629	0122	19.40	0
000079094	ACT	LU2	0475-4 PCT.	0553	0037	6.69	0
000079094	ACT	LU3	2375-5 PCT.	0402	0067	16.67	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 626811 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	ALI		TISSUE	0949	0045	4.74	0
	ALU		TISSUE	1512	0052	3.44	0
000079094	ACT	LI1	0095-3 PCT.	1052	0037	3.52	0
000079094	ACT	LI2	0475-4 PCT.	1024	0037	3.61	0
000079094	ACT	LU1	0095-3 PCT.	1264	0056	4.43	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 629301 DETECTOR TA1537 SPECIES ICRFLO/MOUSE DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0595	0016	2.69	0
	A-C		SOLVENT	0547	0032	5.85	0
	ALI		TISSUE	0518	0036	6.95	2
	ALU		TISSUE	0548	0020	3.65	0
	ACP	LI	AMQ 333 UG/ML	0311	0444	142.77	0
	ACP	LU	AMQ 333 UG/ML	0608	0013	2.14	0
000079094	ACT	LI1	0095-3 PCT.	0501	0070	13.97	0
000079094	ACT	LI2	0475-4 PCT.	0587	0066	11.24	0
000079094	ACT	LI3	2375-5 PCT.	0731	0051	6.98	0
000079094	ACT	LU1	0095-3 PCT.	0561	0053	9.45	0
000079094	ACT	LU2	0475-4 PCT.	0597	0051	8.54	0
000079094	ACT	LU3	2375-5 PCT.	0643	0060	9.33	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 626001 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0954	0277	29.04	0
	A-C		SOLVENT	0862	0246	28.54	0
	ALI		TISSUE	0523	0260	49.71	0
	ALU		TISSUE	0924	0236	25.54	0
	ACP	LI	ANTH 67 UG/ML	0449	0910	202.67	0
	ACP	LU	ANTH 67 UG/ML	0842	0323	38.36	0
000079094	ACT	LI1	0095-3 PCT.	0798	0233	29.20	0
000079094	ACT	LI2	0475-4 PCT.	0477	0180	37.74	0
000079094	ACT	LI3	2375-5 PCT.	0663	0165	24.89	0
000079094	ACT	LU1	0095-3 PCT.	0771	0176	22.83	0
000079094	ACT	LU2	0475-4 PCT.	0524	0192	36.64	0
000079094	ACT	LU3	2375-5 PCT.	0728	0177	24.31	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 627206 DETECTOR TA98 SPECIES ICRFLO/MOUSE DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0657	0031	4.72	0
	A-C		SOLVENT	1003	0031	3.09	0
	ALI		TISSUE	0714	0071	9.94	0
	ALU		TISSUE	0702	0038	5.41	0
	ACP	LI	ANTH 67 UG/ML	0603	0641	106.30	0
	ACP	LU	ANTH 67 UG/ML	0770	0850	110.39	0
000079094	ACT	LI1	0095-3 PCT.	0293	0055	18.77	0
000079094	ACT	LI2	0475-4 PCT.	0388	0140	36.08	0
000079094	ACT	LI3	2375-5 PCT.	0327	0045	13.76	0
000079094	ACT	LU1	0095-3 PCT.	0599	0128	21.37	0
000079094	ACT	LU2	0475-4 PCT.	0361	0039	10.80	0
000079094	ACT	LU3	2375-5 PCT.	0512	0038	7.42	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 629302 DETECTOR TA98 SPECIES ICRFLO/MOUSE DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	ALI		TISSUE	0730	0130	17.81	0
	ALU		TISSUE	0685	0103	15.04	0
000079094	ACT	LI2	0475-4 PCT.	0505	0121	23.96	0
000079094	ACT	LU1	0095-3 PCT.	0570	0126	22.11	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 629202 DETECTOR 00004 SPECIES ICRFLO/MOUSE DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1474	0343	0120	23.27	8.14	0
	A-C		SOLVENT	1304	0341	0104	26.15	7.98	0
	ALI		TISSUE	1244	0330	0108	26.53	8.68	0
	ALU		TISSUE	1194	0345	0103	28.89	8.63	0
	ACP	LI	DMN 90 UM/ML	0848	0574	0232	67.69	27.36	0
	ACP	LU	DMN 90 UM/ML	1111	0360	0127	32.40	11.43	0
000079094	ACT	LI1	0025-1 PCT.	1530	0372	0128	24.31	8.37	0
000079094	ACT	LI2	0125-2 PCT.	1116	0272	0085	24.37	7.62	0
000079094	ACT	LI3	0625-3 PCT.	0902	0160	0064	17.74	7.10	0
000079094	ACT	LU1	0025-1 PCT.	1272	0262	0102	20.60	8.02	0
000079094	ACT	LU2	0125-2 PCT.	1064	0234	0102	21.99	9.59	0
000079094	ACT	LU3	0625-3 PCT.	0900	0221	0091	24.56	10.11	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 627801 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	2500	0518	20.72	0
	A-C		SOLVENT	2288	0591	25.83	0
	ALI		TISSUE	2172	0683	31.45	0
	ALU		TISSUE	2008	0543	27.04	0
	ACP	LI	DMN 90 UM/ML	1419	0876	61.73	0
	ACP	LU	DMN 90 UM/ML	2254	0636	28.22	0
000079094	ACT	LI1	0095-3 PCT.	2166	0697	32.18	0
000079094	ACT	LI2	0475-4 PCT.	2228	0710	31.87	0
000079094	ACT	LI3	2375-5 PCT.	2802	0707	25.23	0
000079094	ACT	LU1	0095-3 PCT.	1678	0600	35.76	0
000079094	ACT	LU2	0475-4 PCT.	2068	0533	25.77	2
000079094	ACT	LU3	2375-5 PCT.	1826	0531	29.08	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 626701 DETECTOR TA1535 SPECIES SPRDAW/RAT DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0909	0098	10.78	0
	A-C		SOLVENT	1063	0108	10.16	0
	ALI		TISSUE	0672	0091	13.54	0
	ALU		TISSUE	0823	0104	12.64	2
	ACP	LI	DMN 90 UM/ML	1272	3140	246.86	0
	ACP	LU	DMN 90 UM/ML	0681	0096	13.97	2
000079094	ACT	LI1	0095-3 PCT.	1063	0382	35.94	0
000079094	ACT	LI2	0475-4 PCT.	1319	0288	21.83	0
000079094	ACT	LI3	2375-5 PCT.	0946	0114	12.05	0
000079094	ACT	LU1	0095-3 PCT.	1097	0062	5.65	0
000079094	ACT	LU2	0475-4 PCT.	1197	0071	5.93	0
000079094	ACT	LU3	2375-5 PCT.	1124	0053	4.72	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 629501 DETECTOR TA1537 SPECIES SPRDAW/RAT DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0600	0025	4.17	0
	A-C		SOLVENT	0641	0017	2.65	0
	ALI		TISSUE	0669	0025	3.74	1
	ALU		TISSUE	0568	0012	2.11	0
	ACP	LI	AMQ 333 UG/ML	0200	0245	122.50	1
	ACP	LU	AMQ 333 UG/ML	0582	0008	1.37	2
000079094	ACT	LI1	0095-3 PCT.	0309	0027	8.74	0
000079094	ACT	LI2	0475-4 PCT.	0311	0025	8.04	0
000079094	ACT	LI3	2375-5 PCT.	0569	0022	3.87	1
000079094	ACT	LU1	0095-3 PCT.	0335	0026	7.76	0
000079094	ACT	LU2	0475-4 PCT.	0477	0020	4.19	0
000079094	ACT	LU3	2375-5 PCT.	0565	0013	2.30	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 626505 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0427	0021	4.92	0
	A-C		SOLVENT	0486	0020	4.12	0
	ALI		TISSUE	0271	0050	18.45	0
	ALU		TISSUE	0374	0028	7.49	0
	ACP	LI	ANTH 67 UG/ML	0269	0588	218.59	0
	ACP	LU	ANTH 67 UG/ML	0250	0683	273.20	0
000079094	ACT	LI1	0095-3 PCT.	0365	0025	6.85	0
000079094	ACT	LI2	0475-4 PCT.	0140	0030	21.43	0
000079094	ACT	LI3	2375-5 PCT.	0126	0019	15.08	0
000079094	ACT	LU1	0095-3 PCT.	0283	0020	7.07	0
000079094	ACT	LU2	0475-4 PCT.	0352	0020	5.68	0
000079094	ACT	LU3	2375-5 PCT.	0511	0035	6.85	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 626502 DETECTOR TA98 SPECIES SPRDAW/RAT DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1712	0272	15.89	0
	A-C		SOLVENT	2147	0267	12.44	0
	ALI		TISSUE	2557	0297	11.62	0
	ALU		TISSUE	1650	0331	20.06	0
	ACP	LI	ANTH 67 UG/ML	1116	0938	84.05	0
	ACP	LU	ANTH 67 UG/ML	1304	0324	24.85	0
000079094	ACT	LI1	0095-3 PCT.	0830	0248	29.88	0
000079094	ACT	LI2	0475-4 PCT.	0996	0262	26.31	0
000079094	ACT	LI3	2375-5 PCT.	1116	0264	23.66	0
000079094	ACT	LU1	0095-3 PCT.	1197	0285	23.81	0
000079094	ACT	LU2	0475-4 PCT.	1417	0286	20.18	0
000079094	ACT	LU3	2375-5 PCT.	1138	0344	30.23	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 629502 DETECTOR 0000D4 SPECIES SPRDAW/RAT DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0886	0381	0191	43.00	21.56	0
	A-C		SOLVENT	0761	0390	0155	51.25	20.37	0
	ALI		TISSUE	0708	0296	0187	41.81	26.41	0
	ALU		TISSUE	0726	0284	0153	39.12	21.07	0
	ACP	LI	DMN 90 UM/ML	0567	0453	0343	79.89	60.49	0
	ACP	LU	DMN 90 UM/ML	0826	0338	0186	40.92	22.52	0
000079094	ACT	LI1	0025-1 PCT.	0633	0295	0099	46.60	15.64	0
000079094	ACT	LI2	0125-2 PCT.	0695	0296	0101	42.59	14.53	0
000079094	ACT	LI3	0625-3 PCT.	0613	0313	0097	51.06	15.82	0
000079094	ACT	LU1	0025-1 PCT.	0684	0277	0101	40.50	14.77	0
000079094	ACT	LU2	0125-2 PCT.	0575	0353	0106	61.39	18.43	0
000079094	ACT	LU3	0625-3 PCT.	0659	0310	0078	47.04	11.84	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 627901 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	1736	0464	26.73	0
	A-C		SOLVENT	1842	0476	25.84	0
	ALI		TISSUE	2708	0814	30.06	0
	ALU		TISSUE	2442	0699	28.62	0
	ACP	LI	DMN 90 UM/ML	1184	0713	60.22	0
	ACP	LU	DMN 90 UM/ML	2430	0752	30.95	0
000079094	ACT	LI1	0095-3 PCT.	2188	0631	28.84	0
000079094	ACT	LI2	0475-4 PCT.	2072	0657	31.71	0
000079094	ACT	LI3	2375-5 PCT.	2278	0696	30.55	0
000079094	ACT	LU1	0095-3 PCT.	2382	0705	29.60	0
000079094	ACT	LU2	0475-4 PCT.	2580	0642	24.88	0
000079094	ACT	LU3	2375-5 PCT.	2412	0759	31.47	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 628001 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	2201	0164	7.45	0
	A-C		SOLVENT	2313	0223	9.64	0
	ALI		TISSUE	2757	0190	6.89	0
	ALU		TISSUE	2683	0203	7.57	0
	ACP	LI	DMN 90 UM/ML	1754	1020	58.15	0
	ACP	LU	DMN 90 UM/ML	2777	0245	8.82	0
000079094	ACT	LI1	0095-3 PCT.	2780	0229	8.24	0
000079094	ACT	LI2	0475-4 PCT.	2795	0186	6.65	0
000079094	ACT	LI3	2375-5 PCT.	2659	0194	7.30	0
000079094	ACT	LU1	0095-3 PCT.	2522	0216	8.56	0
000079094	ACT	LU2	0475-4 PCT.	2702	0195	7.22	0
000079094	ACT	LU3	2375-5 PCT.	2630	0177	6.73	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 629701 DETECTOR TA1537 SPECIES RHESUS/MONKEY DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	2774	0327	11.79	2
	A-C		SOLVENT	1818	0145	7.98	2
	ALI		TISSUE	0981	0181	18.45	2
	ALU		TISSUE	0996	0221	22.19	2
	ACP	LI	AMQ 333 UG/ML	2119	0065	3.07	2
	ACP	LU	AMQ 333 UG/ML	2400	0315	13.13	2
000079094	ACT	LI1	0095-3 PCT.	0877	0198	22.58	0
000079094	ACT	LI2	0475-4 PCT.	0835	0266	31.86	0
000079094	ACT	LI3	2375-5 PCT.	0859	0212	24.68	0
000079094	ACT	LU1	0095-3 PCT.	0824	0235	28.52	0
000079094	ACT	LU2	0475-4 PCT.	0571	0268	46.94	0
000079094	ACT	LU3	2375-5 PCT.	0794	0292	36.78	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104
EXPERIMENT 627104 DETECTOR TA1538 SPECIES RHESUS/MONKEY PROJECT 02468 DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0544	0018	3.31	0
	A-C		SOLVENT	0306	0019	6.21	0
	ALI		TISSUE	0418	0045	10.77	0
	ALU		TISSUE	0554	0021	3.79	0
	ACP	LI	ANTH 67 UG/ML	0334	1940	580.84	0
	ACP	LU	ANTH 67 UG/ML	0674	0026	3.86	0
000079094	ACT	LI1	0095-3 PCT.	0328	0029	8.84	0
000079094	ACT	LI2	0475-4 PCT.	0218	0026	11.93	0
000079094	ACT	LI3	2375-5 PCT.	0162	0025	15.43	0
000079094	ACT	LU1	0095-3 PCT.	0285	0020	7.02	0
000079094	ACT	LU2	0475-4 PCT.	0253	0015	5.93	0
000079094	ACT	LU3	2375-5 PCT.	0213	0006	2.82	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 627401 DETECTOR TA98 SPECIES RHESUS/MONKEY DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1559	0412	26.43	1
	A-C		SOLVENT	1803	0489	27.12	0
	ALI		TISSUE	0916	0518	56.55	0
	ALU		TISSUE	0753	0463	61.49	1
	ACP	LI	ANTH 67 UG/ML	1088	0963	88.51	1
	ACP	LU	ANTH 67 UG/ML	0953	0409	42.92	0
000079094	ACT	LI1	0095-3 PCT.	1469	0435	29.61	0
000079094	ACT	LI2	0475-4 PCT.	1042	0439	42.13	1
000079094	ACT	LI3	2375-5 PCT.	1035	0435	42.03	0
000079094	ACT	LU1	0095-3 PCT.	1345	0406	30.19	0
000079094	ACT	LU2	0475-4 PCT.	1480	0501	33.85	0
000079094	ACT	LU3	2375-5 PCT.	1171	0333	28.44	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 629503 DETECTOR 0000D4 SPECIES RHESUS/MONKEY DATE - 10/27/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0787	0104	0059	13.21	7.50	0
	A-C		SOLVENT	0624	0101	0047	16.19	7.53	0
	ALI		TISSUE	0751	0118	0058	15.71	7.72	0
	ALU		TISSUE	0734	0064	0042	8.72	5.72	0
	ACP	LI	DMN 90 UM/ML	0721	0486	0177	67.41	24.55	0
	ACP	LU	DMN 90 UM/ML	0735	0095	0045	12.93	6.12	0
000079094	ACT	LI1	0025-1 PCT.	0726	0104	0062	14.33	8.54	0
000079094	ACT	LI2	0125-2 PCT.	0778	0111	0061	14.27	7.84	0
000079094	ACT	LI3	0625-3 PCT.	0740	0099	0054	13.38	7.30	0
000079094	ACT	LU1	0025-1 PCT.	0733	0075	0044	10.23	6.00	0
000079094	ACT	LU2	0125-2 PCT.	0691	0086	0049	12.45	7.09	0
000079094	ACT	LU3	0625-3 PCT.	0680	0073	0054	10.74	7.94	0